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## ANNUAL PROGRESS REPORT

Prepared by Dr. Alan DeForest Smith January 25, 1954  
For period 1/1/53 - 12/31/53

Contract: Equipment loan only. NONR 1313 (00)  
Contractor: Trustees of Columbia University, in the City of  
New York

annual rate, does not apply

Principal Investigator: Dr. Gabriel C. Goamini  
(Surgical Pathology, Histochemistry research)  
Associates: Dr. C.A.L. Bassett (Orthopedic Surgery)  
Dr. D.P. Bloch (Histochemistry Research)  
Dr. C. Ragan (Medicine)  
Dr. Karl Meyer (Medicine and Biochemistry)  
Dr. H. Grossfeld (Medicine)  
Assistant: Miss Priscilla Goettles, A.S.

Projects in which equipment on loan from D.W.R. are used:

A 1. Title: Studies on the regeneration of striated skeletal muscle.  
2. Objectives. Studies are made with reference to.  
    a. conditions favoring restitution of experimentally injured muscle in ischaemia, crush and thermal injury.  
    b. cyto- and histochemistry of differentiation in muscle, correlating the presence and distribution of nucleic acids, myohaemoglobin, phosphatases, glycogen and certain oxidative enzymes with the progressive stages of sarcoblastic differentiation.  
    c. modulation, rate of regenerative growth, and mode of nuclear division of skeletal muscle in tissue culture.

B 1. Title: An evaluation of tissue culture as a method of bone graft procurement.  
2. Objectives:  
    a. to determine the requirements for maintenance of potentially osteogenic cells of foetal endosteum in luxuriant proliferation in vitro for extended periods of time.  
    b. to determine the efficiency of freeze-dried cancellous bone (non-viable) and plastic sponge (poly-vinyl) as three dimensional substrates for foetal "osteoblasts".  
    c. to determine the relative effectiveness of freeze-dried cancellous bone and plastic sponge both infiltrated with homogenous foetal "osteoblasts", as *in vivo* bone graft substitute.

C 1. Title: Correlated studies on the chemistry, growth and metabolism of mesenchymal cells of the fibrous and osseous connective tissue, and their products, in mass tissue culture.  
2. Objectives:  
    a. to determine what effects certain steroid hormones exert on fibroblastic growth in vitro.  
    b. to determine which cell types are affected.

- c. to study some of the factors influencing the action of such hormones on cells in culture.
- d. to determine whether acid mucopolysaccharides (m.p.s.) are produced in vitro, and if they are, what kinds and how much.
- e. to discover what controllable factors (composition of medium, pH, oxygen tension, plasma substrate etc.) affect the kinds and amounts of mucopolysaccharides produced in culture.
- f. to study some relationships between m.p.s. (matrix) and reticulum and collagen (fiber) formation in vitro.
- g. to investigate some of the cytochemical characteristics of cells associated with m.p.s. and fiber formation in vitro.
- h. to gain information concerning intermediate stages of m.p.s. formation, and to study some of the enzyme systems concerned in the process.

#### Summary of results and Plans

Project A. Ischaemia of the tibialis anticus muscle of rabbits, produced following the method of Le Gros Clark (1) was found to result in an infarct which was repaired by extensive new growth of muscle. Compared with traumatic injuries, it was confirmed, as other authors had suggested, (2, 3,) that restitution of striated skeletal muscle depends upon the preservation of patent uninterrupted sarcolemmic endomysial guideplanes into which continuous sarcoblastic slips can grow, and upon the restoration of vascular competency. Mature muscle, although considered a highly specialized tissue type, was found to have a very great potential for proliferation and growth, modulation and redifferentiation, facts to which our experience with adult muscle in tissue culture (4) gives definite support.

The mode and rate of muscle growth in vitro have been recorded, and correlated with the regenerative growth which ensues after ischaemic and relatively mild thermal injury to muscle in rabbits and rats. Sarcoblastic development and rate of growth in tissue culture was found to be almost identical with the earlier stages of regeneration in vivo. The problem of the origin of the free histiocytoid cells in the sarcolemmic tubes which form after severe muscle injury (5) (and also the origin of some of the cellular forms seen in supposedly myogenic tumors) was elucidated with the help of tissue culture of mature muscle. It was found that sarcoblastic (regenerating muscle) ribbons were capable of budding-off free motile mononucleate cells, capable of mitotic division and phagocytosis, confirming by direct recorded observation earlier studies on foetal muscle in vitro (6). The fate of these modulated elements and differentiation of muscle in vitro will be studied.

Extensive anatomical studies have been conducted on the lesions produced by ligation of nutrient arteries of a muscle, and on injuries consequent on application of heat, dry ice and on crushing muscle. The changes have been described, and it has been confirmed that the biological potentiality of mature differentiated striated skeletal muscle to proliferate and regenerate is very great. The most important single factor determining the success

of the restitutive process is the integrity of the sarcolemmal or endomyseal planes which serve as frameworks. Should these be destroyed or collapse (as in crushing, severe burns) regeneration is abortive.

The sarcoblasts are being studied histochemically to correlate the chemical and morphological changes which appear in successive steps in differentiation. Preliminary studies indicate that the cytoplasm of the sarcoblast is rich in RNA ribonucleotide and in phosphatase prior to and during the first visible appearance of myofibrils and definitive positive birefringence. These substances diminish with differentiation, and are hardly apparent after cross-striations and acidophilia occur. Lower nucleotides are augmented however, as the fiber matures.

Future plans call for quantitative biochemical determinations of the rate of accumulation of nucleic acid in regenerating muscle, to be related to parallel histochemical work. Identification (bio and histochemical) and localization of thiols, glycogen, phosphatases and oxidative enzymes in regeneration will be undertaken.

Intensive study has also been made of the amitotic nuclear partition which is the rule in muscle. Anatomic observations with periodic photography of living sarcoblasts in culture are now being made. Microspectrophotometric analyses to date have indicated that in spite of rapid non-mitotic division, the daughter nuclei receive diploid equivalents of DNA.

Project B. The experimental methods have been given in an application for a Grant In Aid submitted by Dr. C.A.L. Bassett, now pending in the O.N.R. For details reference should be made to this application.

To date, it has been verified by means of tissue culture that exposure of human cancellous bone to air for periods up to several hours, did not adversely influence its viability. Frozen and dehydrated bone, however, showed no signs of viability.

Cells derived from human endosteum (cancellous bone) are being cultivated over a long term in preparation for obtaining a race of human cells of osteogenic potentiality for mass culture. This involves patient and repeated subculturing.

The types of cells present are being studied morphologically and histochemically in an attempt to identify and differentiate the osteoblast, i.e. that modulated form of mesenchymal cell capable of deposition of specific bone matrices.

Project C. a - c. It is known that cortisone affects fibroplasia in vivo (7), but the reports of its direct action on fibroblasts in vivo have so far been conflicting or inconclusive.

It has been found that fibroblasts grown in a medium of amniotic fluid and plasma without the customary embryonic extract (EE) provided the best means of measuring the effect of the steroid hormones on growth. With water-soluble hydrocortisone in concentrations of 200 mcg/ml or greater, distinct inhibition of fibroblast growth was demonstrated. Growth of epithelium was not affected. In the presence of EE, inhibition could be shown only with higher concentrations of hydrocortisone, suggesting that EE contains a principle antagonistic to the fibroblast-inhibiting effect of

hydrocortisone. Cortisone acetate in aqueous suspension used in the same concentration caused only slight inhibition of growth, while hydrocortisone in suspension caused considerably greater inhibition of growth than did cortisone acetate, but about one-third as much inhibition as water solution hydrocortisone. Desoxycorticosterone, both in suspension and solution was found to be as potent an inhibitor of fibroblastic proliferation as soluble hydrocortisone.

In every case, the effect of the steroid was reversible, and normal growth was resumed after the cells were restored to a drug-free medium. A profound direct effect of a cortisone was thus unequivocally shown *in vivo*.

d - h. In order to gain basic information on the formation of the matrices of connective tissue and bone, to serve in an understanding of their changes in disease, injury and repair, the activities of an isolated system composed almost entirely of the growing mesenchymal cells of fibrous, synovial or endosteal tissue affords an advantageous object of study. Cultures of such cells should be grown under controllable conditions, and must be capable of yielding several hundred ml. of fluid medium and several grams of cells to permit chemical analysis. Considerable effort has been expended in the arrangement of facilities and invention of methods adapted to the purposes described. Bacteriological culture dishes, permitting mass growth of cells over large surfaces while allowing microscopical observation of the outgrowth have been devised after many experiments. A method of mass tissue culture employing media whose base is amniotic fluid has been elaborated.

Using chick heart and human foetal skin, mass tissue cultures under conditions of optimal growth have been maintained for 4 - 5 weeks, and submitted for chemical analysis. While the chemical analytic work is still in its preliminary stages, it may be stated that abundant mucin-clots, increasing in quantity with the age of the cultures, have been found in the supernatant material. These, and the cellular components, are being subjected to analytic study.

Future plans call for implementation of the stated objectives of this research. Using simplified media or amniotic fluid and small amounts of embryo extract or its dialysate, attempts will be made to influence m p's production by making available carbohydrate moieties, sulfated compounds, or by adding drugs.

Histochemical work shows that there is intense intercellular metachromasia in the outgrowth zone in cultures of fibroblasts derived from human skin and umbilical cord. The histochemical characteristics of fibroblasts engaged in m p's production and their relation to fiber formation will be studied after histochemical identification of the metachromatic material. The phenomenon of vital metachromasia seen in living cells on the uptake of basic dyes of the thiazine group (e.g. toluidine blue) is also being studied.

Additional note: Equipment presently in use in this laboratory under an equipment-loan contract with the Office of Naval Research, was originally assembled under contract N6 ORI-110 Task Order II with the Office of Naval Research. This property formed the basic equipment of the Connective Tissue Laboratory of the Department of Orthopedic Surgery, under the principal direction of Dr. S.S. Hudeck.

Subsequent to July 1951 the Connective Tissue Laboratory was continued in the charge of Dr. J.W. Blunt, Jr. The Naval property was retained under an equipment-loan contract negotiated with Dr. Alan DeForest Smith, Director of the New York Orthopedic Hospital and the Orthopedic Surgery Department, under those egis the laboratory continued to function.

In July 1952, on the advice of a special committee of the College of Physicians and Surgeons (including Drs. R. Lattes, A.P. Stout, A. DeF. Smith, K. Meyer, M. Murray, C. Ragan, and E. Sproul) the preexisting "Connective Tissue Laboratory" was reorganized to enlarge the scope of contemplated investigations.

Some of the basic and necessary permanent equipment for the Histochemistry Laboratory, now housed in Rooms 216 to 220 of the Institute of Cancer Research in the College is loaned from the O.N.R.

Operating expenses are met entirely from grants other than government sources. The laboratory has had no O.N.R. grant during the period covered in this report.

Attention is also drawn to the report submitted by the staff of this laboratory to the O.N.R. on Sept. 28, 1953 to cover the period up to Sept. 15, 1953.

#### Publications and Reports

1. Godman, G. and Murray, M.R., Influence of colchicine on the form of skeletal muscle fibers in tissue culture. P.S.E.B.M. 84:668, 1954.
2. Godman, G. Lecture: Anatomical factors in the regeneration of striated muscle. New York Orthopedic Hospital, June 2, 1953.
3. Bloch, D. Effect of colchicine on synthesis of DNA in tissue cultured rat fibroblasts. P.S.E.B.M. 84:341, 1953.
4. Grossfeld, H. A method of mass tissue culture using bacteriological culture dishes. In press.
5. Grossfeld, H., and Ragan, C. The action of cortisone on cells in tissue culture. I. Effect on cell growth. In preparation.

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